Progress in HIV vaccine development

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Recent advances in HIV vaccine development include initiation of the first efficacy trials and substantial expansion of the preclinical pipeline. Several preclinical candidate vaccines have induced strong cellular immune responses and provided impressive protection against AIDS in non-human primate models; however, candidates that induce broadly neutralizing antibodies remain elusive.

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Abbreviations

CTL	cytotoxic T lymphocyte
HIV	human immunodeficiency virus
MHC	major histocompatibility complex
SIV	simian immunodeficiency virus
TCLA	tissue-culture laboratory-adapted

Introduction

A safe and effective vaccine is the best hope for stopping the spread of HIV worldwide. As the 20th anniversary of the discovery of HIV approaches, considerable optimism is building that identification of an HIV vaccine is within reach. Advances in vaccine design, animal models and clinical research have recently converged to create a promising pipeline of candidate vaccines. However, overcoming remaining scientific, logistical and financial challenges will require the talents and resources of all stakeholders — academic researchers, pharmaceutical companies, philanthropic organizations, governments and communities. This review outlines the major scientific advances of the past two years and highlights important challenges in converting the current optimism into success.

Clinical trial results

The HIV envelope is the predominant target of neutralizing antibodies in HIV-infected individuals. Several adjuvanted recombinant monovalent HIV envelope proteins (e.g. gp160 or the mature exterior portion gp120), based on tissue-culture laboratory-adapted (TCLA) isolates of subtype B HIV, have been extensively studied in human trials. These candidates induced neutralizing antibodies in virtually all volunteers tested, but these antibodies exhibited little cross-reactivity against primary isolates of HIV [1]. Subsequently, bivalent candidates developed by VaxGen Inc. (AIDSVAX®, Brisbane, CA) have advanced to efficacy trials in the USA and Thailand (Table 1). The bivalent vaccine comprises two gp120s, one from a subtype B TCLA isolate of HIV and one from a subtype B or E primary isolate, and trial results are expected around the end of 2002.

Until recently, the frequency and strength of neutralizing antibodies and cytotoxic T lymphocytes (CTLs) induced by peptides based on the viral envelope or internal proteins have been disappointing. Peptide lipid-ation has shown some promise in improving immunogenicity — lipopeptides derived from env, gag and nef proteins induced CTLs to one or more peptides in up to two thirds of immunized volunteers [2]. Use of novel adjuvants, cytokines and co-stimulatory molecules are also under investigation. For example, a saponin adjuvant (QS21), although not well tolerated, decreased the dose of gp120 required to induce high-titer antibodies [3•]. Peptides based on predictions of epitopes representing immunodominant, conserved, 'supertype'

Table 1

HIV vaccine candidates in clinical trial.

Vaccine	HIV subtype	Producer	Current status
gp120	B/B, B/E	VaxGen	Phase III trials ongoing in the US and Thailand
ALVAC-HIV	B, E	Aventis Pasteur	In phase II trials in the US, Haiti, Brazil and Trinidad (subtype B), and Thailand (subtype E); tested alone or in combination with gp120
ALVAC-HIV	A	Aventis Pasteur	Ready for phase I trial in Uganda
Lipopeptides LP5, LP6	B	ANRS	In phase I trials in France
Vaccinia TBC-3B	B	Therion	In phase I trials in the USA
DNA-HIV	B	Apollon	Phase I trials completed
DNA-HIV, MVA-HIV	A	University of Oxford	In phase I trials in the UK and Kenya
NYVAC-HIV	B	Aventis Pasteur	Ready for phase I trial in the USA
DNA-HIV, Adenovirus-HIV	B	Merck	In phase I trials in the USA

ALVAC-HIV, recombinant canarypox expressing multiple HIV genes; ANRS, National Agency for AIDS Research, France; MVA-HIV, modified vaccinia Ankara, an attenuated vaccinia vector, expressing multiple HIV genes; NYVAC-HIV, an attenuated vaccinia vector expressing multiple HIV genes; TBC-3B, attenuated vaccinia vector expressing multiple HIV genes.

Table 2

Candidate vaccines in preclinical development.

Vaccine*	HIV subtype	Preclinical partners(s)†
Adeno-associated virus expressing multiple genes	C	Targeted Genetics, Ohio State University, IAVI
Adenovirus expressing multiple genes (replicating)	B	NCI
ALVAC expressing multiple genes	A	Aventis Pasteur, WRAIR
DNA and adenovirus (replicon) expressing multiple genes	B	Merck
DNA and adenovirus expressing novel *gag-pol* and novel *env*	B	NIAID Vaccine Research Center
DNA and MVA expressing multiple genes	B, A/G	Emory University, NIAID,CDC
MVA expressing multiple genes	A, D	WRAIR
DNA, Sindbis replicons expressing multiple genes, novel recombinant envelope proteins	B, C	Chiron, NIAID
DNA expressing multiple HIV genes, DNA expressing cytokine gene and peptide boost	B	Wyeth-Lederle, NIAID
DNA and fowlpox expressing multiple HIV genes and cytokine	B, E	University of New South Wales, NIAID
DNA-*env* and envelope protein	Multiple	ABL, NIAID
Gp120 and regulatory proteins in novel adjuvants	B	GlaxoSmithKline
MVA expressing multiple genes, including CCR5-using envelope	B	Therion, University of Massachusetts, NIAID
MVA, NYVAC, DNA, Semliki Forest Virus expressing multiple genes and envelope protein	C	Eurovac, Aventis Pasteur
P55 VLP	B	Protein Sciences, NIAID
Salmonella expressing multiple genes	A, A/G	IHV, IAVI, NIAID
Vaccinia-*env* and envelope proteins	Multiple	St Jude, NIAID
VEE-*gag* (replicons)	C	IAVI, NIAID
VEE expressing multiples genes (replicons)	C	NIAID, WRAIR

*CCR5, CC chemokine receptor 5; MVA; modified vaccinia Ankara; NYVAC, attenuated vaccinia virus; VEE, Veneuzuelan equine encephalitis virus; VLP, virus-like particles. †CDC, Centers for Disease Control and Protection; Eurovac, consortium of 21 laboratories in 8 European countries funded by the European Union; IAVI, International AIDS Vaccine Initiatives; IHV, Institute of Human Virology; NCI, National Cancer Institute; NIAID, National Institute of Allergy and Infectious Diseases; WRAIR, Walter Reed Army Institute of Research.

epitopes (e.g. recognized by multiple alleles) are also under development [4•].

DNA candidates, thus far, have not fulfilled the expectations arising from early studies in mice. DNA vaccines encoding *env* and *gag-pol* genes were safe in doses of up to 3 mg, but failed to induce strong immune responses (Goepfert P *et al*., *Int Conf AIDS* 1998,12:635) [5•]. Codon-optimized, adjuvanted and particle-formulated candidates are expected to perform better.

Live recombinant vectors expressing one or more HIV genes are among the most promising candidate vaccines. The first HIV recombinant viral vector evaluated in humans was an attenuated vaccinia that expressed the HIV gp160 envelope protein. Subsequent trials evaluated a more complex vaccinia recombinant expressing *env* and *gag-pol* genes (Keefer MC *et al*., *Int Conf AIDS* 1998,12:278). Recipients developed neutralizing antibodies but CTL induction was limited; however, sensitive assays to detect CTL responses were not available at that time.

The potential virulence of vaccinia in immune deficient individuals has directed attention to recombinant viral vectors with very limited or no ability to replicate in human cells and to replicons, which lack the full complement of genes required for complete replication and/or particle formation. The most extensively studied vector in human trials is ALVAC®, a recombinant canarypox developed by Aventis Pasteur. Five canarypox-HIV recombinants, alone or in combination with gp120 subunit vaccines, have been evaluated in humans. Although HIV-specific CTL responses were detected in only about one-third to one-half of volunteers, the concomitant induction of neutralizing antibodies and T-helper responses in volunteers boosted with gp120 has made this 'prime-boost' a promising approach [6••,7••]. A phase II study of a canarypox HIV candidate (ALVAC® vCP1452) in combination with gp120 (AIDSVAX®B/B, VaxGen) is underway in the USA. This study will lead to an efficacy trial in late 2002 if immunogenicity criteria are met. Another recombinant pox vector, modified vaccinia Ankara (MVA, IDT Germany, under contract to the University of Oxford, UK) expressing HIV gag and a number of CTL epitopes, has recently entered clinical trial in the UK and Kenya.

Preclinical studies

Preclinical studies have truly fueled the current optimism. First, several candidate vaccines have produced promising results in rather stringent non-human primate models of AIDS. Second, the number of candidates advancing toward phase I human trials has increased dramatically in the past three years (Table 2).

Advancements in the field of HIV and SIV (simian immunodeficiency virus) immunology have permitted more thorough and sensitive evaluation of cellular responses to HIV and SIV candidate vaccines (Table 3). Until a few years ago, cellular immune assays were limited to measuring proliferation of T cells exposed to antigen

Table 3

Laboratory assessments of HIV–vaccine-induced immune responses.

Type of response	Assessment	Specific assays
Humoral immune responses	Antibody binding assays	ELISA, Western blots
	Antibody neutralization assays	
	Antibody-mediated fusion inhibition assay	
	Antibody-dependent cytotoxicity	
Cellular immune responses	Proliferation to soluble antigens (mostly $CD4^+$ cells)	
	Cytotoxicity	Chromium-release assay
	Enumeration of antigen-specific T cells	Tetramer binding
	Enumeration of cytokine-producing cells (IFN-γ, TNF-α, etc.)	ELISPOT, intracellular staining (flow cytometry)

ELISA, enzyme-linked immunosorbent assay; IFN-γ, interferon-γ; TNF-α, tumor necrosis factor α.

and CTL-mediated killing of autologous cells expressing HIV epitopes, both of which are subject to considerable variability. Newer assays such as ELISPOT — an enzyme-linked immunosorbent assay (ELISA) format — allow for detecting and counting cells producing interferon-γ or other cytokines in response to specific peptides [8••]. T cells that recognize specific peptides bound to major histocompatibility complex (MHC) class I molecules can also be detected and counted by flow cytometry using tetramers, which are molecules consisting of four copies of a given class I molecule bound to their cognate peptide and alkaline phosphatase [9]. These advances, combined with identification of CTL epitopes and their restricting MHC class I molecules in rhesus macaques, have made more detailed dissection of vaccine-induced immune responses in immunized animals feasible [10–12,13•,14•].

Several candidate vaccines have been shown to protect rhesus macaques from disease following challenge with a highly pathogenic virus weeks to months after the last immunization [14•,15–18,19••,20••,21]. Immunized animals became infected but controlled the infection to the extent that, in some cases, viral levels in the blood were low to undetectable, $CD4^+$ T-cell counts remained stable, and the animals did not progress to disease months after most if not all control animals progressed to disease [19••,20••]. Protection correlated with strong vaccine-induced immune responses mediated by $CD8^+$ T cells. This is somewhat reminiscent of highly exposed Kenyan sex workers whose resistance to detectable HIV infection was associated with HIV-specific $CD8^+$ T-cell responses [22]. Interestingly, a small number of these women who had left or decreased their sex work became HIV infected, demonstrating that their protection was not life-long and suggesting that continued exposure to antigenic stimulation may be required to afford long-term protection [23]. Thus, long-term follow-up of experimental animals will be required to determine whether these 'protected' macaques will eventually lose their controlling immune responses and progress to disease and to what extent boosting of the immune system may be necessary.

Transmissibility of virus from these animals has also not yet been determined, although viral load in the plasma of HIV-infected persons strongly correlates with transmission to sexual partners and to newborns [24••,25]. Whether vaccine-induced long-term control of HIV replication will prevent HIV transmission remains to be determined.

Most candidate vaccines that controlled infection through strong cellular immune responses did not induce high-titer neutralizing antibodies. However, cocktails of antibodies passively transferred have protected macaques against pathogenic challenge — protection correlated with *in vitro* neutralization results [26,27••]. Studies with strains of HIV that have been genetically modified have provided additional evidence that antibody can contribute to the control of viremia [28]. Thus, a candidate vaccine that induces broadly neutralizing antibodies as well as strong cellular responses could provide improved protection against infection or disease.

Additional optimism has also come from the substantial increase in the number of vaccine candidates that are scheduled to enter clinical trial in the coming 1–3 years. In view of the high risk and relatively poor global market forces that dissuade aggressive private sector investment in product development, particularly for candidate vaccines based on HIV subtypes that predominate worldwide, government and philanthropic sources have supported the preclinical development of many of these (Table 2).

Vaccine design

As noted above, recombinant monomeric gp120 envelope candidate vaccines elicit antibodies that are generally subtype specific and neutralize TCLA isolates but few if any primary isolates of HIV. For this reason, there is little confidence that the candidate recombinant envelope vaccines now in clinical trials will induce neutralizing antibodies with the breadth necessary for worldwide use. At a minimum, cocktails of gp120s would be necessary. Antibodies induced in human volunteers by ALVAC and gp120 have been reported to neutralize five out of 14 primary isolates of HIV, including HIV with different co-receptor usage [29•].

Unfortunately, although new viral vectors that enter human trial this year and next may prove to induce more consistent or higher levels of CTLs, candidates likely to induce broadly neutralizing antibodies have not yet been identified.

Efforts to design a vaccine that induces broadly reactive antibodies against primary isolates were given a boost with the report that a fused cell preparation (comprising cells expressing HIV envelope and cells expressing HIV receptors) induced antibodies in transgenic mice that neutralized 23 of 24 primary isolates from different HIV subtypes [30]. Although this result has not been reproduced, several groups have constructed modified envelopes that might reveal conserved conformational epitopes critical to HIV entry with somewhat encouraging results. The V2 loop is one of three highly variable sequences of the HIV envelope. Removal of this from a DNA vaccine resulted in a candidate vaccine that induced antibodies that were somewhat more broadly reactive than the parent molecule [31]. A stabilized envelope trimer, designed to resemble the functional envelope glycoprotein on the surface of the virion, induced neutralizing antibodies against select primary isolates and TCLA HIV, whereas trimers derived from TCLA HIV induced antibody that neutralized only the homologous virus [32]. Other approaches — including stabile oligomerization, removal of carbohydrate molecules, modification of envelope to be independent of CD4, and gp120–CD4 fusion proteins or complexes — are also under investigation [28,33•,34•,35–37]. No outstanding envelope candidate has yet emerged.

Other remaining challenges

One achievement that would advance the field of HIV vaccine development more than any other would be identifying a candidate vaccine that shows some protection in human trials and determining the immune correlate(s) — the type, magnitude, breadth and/or location of immune responses — that are associated with protection. Sensitive and quantitative antibody assays have been in existence for decades. The new cellular assays described above are now being employed in vaccine clinical trials, increasing our ability to detect and quantify vaccine-induced cellular responses. This has improved hope that an immune correlate can be identified in the context of large efficacy trials.

Table 4

Possible outcomes of immunization against HIV.

Outcome	Specific effects
Sterilizing immunity	No cells contain integrated provirus (no virus detected at any time in blood, lymph nodes, or at the site of exposure using the most sensitive PCR assay) No seroconversion to HIV proteins not in the vaccine No CTLs to HIV proteins not in the vaccine
Transient infection	Low level of virus detected only very early following exposure (no virus detected in blood, lymph nodes, or at the site of exposure using the most sensitive PCR assay at 6 months and all later times) No or transient seroconversion to HIV proteins not in the vaccine No or transient CTLs to HIV proteins not in the vaccine
Controlled infection	Virus levels fall to and remain at low to undetectable levels (<1000 RNA copies/ml) following the acute stage of infection Seroconversion to HIV proteins not in the vaccine occurs CTLs to HIV proteins not in the vaccine are present
Lack of transmission to others	Virus levels in blood and secretions remain insufficient to infect others

Various potential outcomes might result from immunization (Table 4). Because HIV integrates into the host cell's DNA, once infection occurs, it may not be possible to completely eliminate the virus. Long-term control may be the only feasible outcome. In any case, for a vaccine to have substantial public health value it should prevent the vaccine recipient from passing the virus on to others. Evaluating outcomes other than sterilizing immunity, defined as the absence of detectable infection, will require long-term follow-up and will present enormous challenges.

Another challenge is to decipher the relevance of different HIV subtypes to vaccine development. Several studies have demonstrated that antibody recognition does not correlate completely with genetic subtype [38,39]. Further, CTLs directed against one HIV subtype can kill cells

Table 5

Challenges to conducting preventive HIV vaccine efficacy trials.

Industrialized countries	Developing countries
Relatively low incidence of HIV infection even in higher risk groups requires large trials of thousands per arm	Concerns regarding exploitation and unequal partnerships
At-risk populations present recruitment and retention challenges particularly women at sexual risk, men at heterosexual risk and intravenous drug users	Concerns that the country will not have affordable access to the vaccine if proven efficacious
Distrust of researchers and government	Infrastructure needs: clinics, labs, equipment, supplies
Growing misunderstandings and distrust of vaccines in general	Training needs: science, good clinical practice, ethics, lab assays, data management
	National authorities and institutional review boards poorly supported or nonexistent

infected with HIV from other subtypes, due largely to the more highly conserved nature of the internal HIV proteins. The pattern of such cross-killing varies and is less efficient relative to homologous targeting against cells infected by HIV from the same genetic clade; however, the magnitude needed to provide protection remains unknown [24••,40,41]. In addition, individuals with different human leukocyte antigen (HLA) backgrounds are likely to focus CTL responses on different epitopes, which could theoretically impact immune responses to vaccines and efficacy of vaccines found to be effective in other populations [42]. Until a correlate of immune protection is validated, clinical trials must be carried out in multiple countries, where different HIV subtypes circulate, to determine whether any vaccine will be broadly efficacious. Some of the problems associated with conducting such trials are shown in Table 5.

Conclusions

With the advent of improved cellular immune assays, there is a strong desire to move candidate vaccines that could prove at least partially effective into efficacy trials to attempt to define immune correlates. However, as the properties required in a successful HIV vaccine remain unknown, academic creativity in the design of vaccines, animal models and clinical trials is needed. This should ensure that improvements would continue if the candidate vaccines in trials or in the pipeline prove lacking in the degree, breadth or durability of efficacy. Fortunately, in recent years a number of promising new candidate vaccines that induce strong cellular immune responses have yielded improved results in preventing AIDS in animal models. Several of these candidates have recently or will soon enter clinical trials, fueling the current optimism that identifying a safe and at least partially effective HIV vaccine in this decade is an achievable goal.

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